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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/741,426	12/21/2000	Akintade Oyedele Dare	MHK-099	5247

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EXAMINER
GOLDBERG, JEANINE ANNE

ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 02/22/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/741,426	DARE, AKINTADE OYEDELE	
	Examiner	Art Unit	
	Jeanine A Goldberg	1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 December 2000* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-17* is/are pending in the application.

4a) Of the above claim(s) *12,13,16 and 17* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11,14 and 15* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

   I. Claims 1-11, 14-15, drawn to a method of assaying DNA for abasic sites using ARP, methods of quantitatively assaying DNA damage, and a method of assaying repair capacity of sample DNA, classified in class 435, subclass 6.

   II. Claims 12-13, drawn to a kit for assaying sample DNA, classified in class 435, subclass 6.

   III. Claim16-17, drawn to an apparatus that automates assaying of DNA, classified in class 435, subclass 287.2.

2. The inventions are distinct, each from the other because of the following reasons:

A) Inventions I and II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product (MPEP § 806.05(h)). In the instant case the kit (product) may be used in materially different methods from the method of Group I, for assaying DNA such a methods of diagnosing Alzheimer's disease or other diseases which rely on abasic sites. The method may be practiced with another materially different product, which does not require a surface treatment solution, or a washing detergent.

B) Inventions I and III are related as process and apparatus for its practice. The inventions are distinct if it can be shown that either: (1) the process as claimed can be practiced by another materially different apparatus or by hand, or (2) the apparatus as claimed can be used to practice another and materially different process. (MPEP § 806.05(e)). In this case the method may be practiced by another materially different apparatus or by hand. The method does not require automation of any nature. Alternatively, the apparatus may be used in methods of diagnosing disease by determining high levels of abasic sites.

C) The kit claims of Group II and the apparatus claims of Group III are directed to different products.

3. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by the different classifications and their divergent subject matter, restriction for examination purposes as indicated is proper.

4. During a telephone conversation with Lawrence Harbin on November 5, 2001 a provisional election was made without traverse to prosecute the invention of Group 1, claims 1-11, 14-15. Affirmation of this election must be made by applicant in replying to this Office action. Claims 12-13, 16-17 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Information Disclosure Statement

5. The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

The list is found on pages 18-20 of the specification.

Specification

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01.

Page 9 contains a hyperlink.

Priority

6. This application claims priority to provisional application 60/171,309, filed December 21, 1999.

It is noted that the examiner could not find basis for the claims in the provisional application. However, the examiner invites the applicant to point to support, in the event

applicant believes that the application should receive benefit of the December 21, 1999 date.

Claim Rejections - 35 USC § 112

7. Claim 11 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claim is drawn to a method which is directed to detecting abasic sites in very low concentration being in the range of 1-10 ng/mL.

The specification teaches detection of abasic sites in 10ug/mL, 5ug/mL and 2.5 ug/mL (Figure 2). The specification does not teach any detection of abasic sites in concentrations as low as 1-10 ng/mL. 10ug/mL is equivalent to 10,000ng/mL.

The art teaches sensitive and specific assays which detect abasic sites in two hundred micro liters of calf thymus DNA containing AP sites which are at a concentration of 10ug/mL.

Neither the specification nor the art teaches how to make and use the invention as broadly as claimed. Neither the specification nor the art teaches how to allow sensitive detection on such a magnitude. The concentration of DNA is so low within the solution is unpredictable that any results will be obtained. As shown in Figure 2, 2.5 ug/mL of DNA was very small at 1 AP site/10,000 bp.

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 1-11, 14-15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) Claim 1-6 are indefinite over the recitation "the abasic sites", Claim 1, step (ii). The claim appears to be directed only to abasic sites in the control DNA. Therefore, it is unclear whether the method is directed to only detecting "the abasic sites" in the control DNA having known abasic sites or whether the claim was intended to be drawn to a method of reacting the sample DNA and control DNA with an aldehyde group-specific reagent, namely ARP. Furthermore, the claim appears to include a Markush group, "reagent selected from a group of reagents including...". This is not a Markush group because there is only one element in the list. It is unclear whether applicant is intending to encompass additional aldehyde group-specific chemical reagents or just ARP.

B) Claim 2 is indefinite because it is unclear what is meant by "tagged or labeled separately with a the residue of the ARP reagent". It is unclear whether there is a typo in the claim or whether there are missing words. Clarification is requested.

C) Claim 6 lacks clear antecedent basis. Claim 6 does not provide a "labeling step". Claim 1 appears to contain a "binding step", a "reacting step, and a "detecting step". Claim 1 however, does not appear to contain a labeling step.

D) Claim 7 is indefinite because it is unclear what the clauses are modifying. For example, the claim is directed to a binding step which binds to an analysis plate sample DNA and multiple control DNA specimens each having of known number of abasic sites. First, it appears as though the claim contains an extra word, namely "of". Secondly, it is unclear whether each DNA, sample and control DNA contains a known number of abasic sites or whether just the control DNA has a known number of abasic sites. Furthermore, in the "performing an ELISA-like method" step, the claim recites to obtain "one of absorbance, optical density and color density of the sample and control DNA". It is unclear what "obtain one of absorbance, optical density and color density of the sample and control DNA" means. In the event that applicant intended that one of the measurements is required, clarification is requested.

E) Claims 8-9 are indefinite over the recitation "performing an ELISA-like method to obtain one of absorbance, optical density and color density of the sample and control DNA". It is unclear what "obtain one of absorbance, optical density and color density of the sample and control DNA" means. In the event that applicant intended that one of the measurements is required, clarification is requested. Similarly, in the final comparing step "comparing at least one of color, optical density and absorbance" is unclear. Furthermore, the preamble states that the method is a method of assaying repair capacity of sample DNA, however the final process step is a comparing step which determines relative enzyme activity levels of the sample and control DNA. Therefore, it is unclear whether the method is a method of assaying repair capacity of the sample DNA or whether the method is a method of comparing color, optical density or

absorbance, or even determining relative enzyme activity level. It is unclear how the final process step relates to the method of assaying repair capacity. Thus, the final process step does not meet the preamble.

F) Claims 10-11 are indefinite because it is unclear what the final process step (vi) is intending to claim. It is unclear whether the abasic sites are quantitatively assessed or rather the quantity of DNA is assessed. Since the preamble does not provide any guidance, the final process step is unclear.

G) Claim 11 is indefinite because it is unclear whether Claim 11 is adding an additional step to Claim 10 or rather is meant to limit one of the already provided steps. For example, it is unclear whether Claim 11 is directed to further binding to the analysis plate a high percentage of DNA within a solution of relatively low concentration. Furthermore, it is unclear what the claim is intended to mean since the claim calls for a relatively high percentage of DNA contained in a solution of relatively low concentration. It is unclear how the percentage can be both high and have low concentration. Percentage is related to concentration. Clarification is requested.

H) Claims 14-15 are directed to a method of determining DNA repair capacity. However, the final process step is determining the number of abasic sites remaining on the analysis plate after the enzyme reaction whereby to assay the ability of the cell to undergo DNA repair. It is unclear how the method of determining DNA repair capacity is related to the number of abasic sites remaining. There does not appear to be any comparison between the DNA prior to treatment and following treatment with the repair enzyme. Moreover, the recitation "determining the resulting number of abasic sites

remaining on the analysis plate after the enzyme reaction whereby to assay the ability of the cell to undergo DNA repair" is confusing. This appears to be two independent thoughts which are not linked.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

9. Claims 1, 3, 6-9, 14-15 are rejected under 35 U.S.C. 102(b) as being anticipated by Kubo et al (Biochemistry, Vol. 31, pages 3703-3708, 1992).

Kubo et al (herein referred to as Kubo) teaches a "novel, sensitive, and specific assay for abasic sites, the most commonly produced DNA lesion". Kubo teaches the use of a biotin-tagged reagent specific for the aldehyde groups, called Aldehyde Reactive Probe (ARP). Kubo teaches that "after modification of the aldehyde group with ARP, the biotin-tagged Ap site can then be easily quantitated by the use of avidin/biotin complex technology in an ELISA-like microtiter plate assay" (page 3703, col 2). Kubo teaches a method of binding to an analysis plate (Immulon I microtiter plates) both

sample DNA and control DNA (calf thymus DNA containing AP sites) having known abasic sites (page 3704, col 2). The plates were then washed and ARP reagent was added. Unbound ARP reagent was removed by washing with PBS-Tween (page 3704, col 2). Finally, ABC was added to each well, washed and horseradish peroxidase substrate was added and the color development was stopped after an appropriate time (limitations of Claim 6). Absorbance at 490 nm was taken and standard curves were determined with either f1 or calf thymus DNA containing known amounts of AP sites (limitations of Claims 1 and 3). Table 1 illustrates the specificity of the ARP reagent and illustrates that the absorbance is greater with the greater number of AP sites. Kubo clearly teaches "microtiter plates coated with calf thymus DNA containing each of these lesions were incubated with the ARP reagent, and the mount of biotin bound in each of the wells was measured by enzyme assay using the avidin/biotin complex (ABS) conjugated with horseradish peroxidase" (Page 3705, col 1).

With respect to Claims 8-9, 14-15, Kubo teaches an additional method for determining the number of intermediary AP sites produced by the action of endonuclease III. The method comprised using 200 ul of calf thymus DNA containing different amount of thymine glycol which was added to each of the wells of a microtiter plate, incubated and washed. Then endonuclease III was added and the plates were incubated. The reaction was terminated and ARP reagent was added and the number of AP sites was determined. The results are illustrated in Figure 4.

The ARP assay is rapid, simple, specific and sensitive and offers the possibility of processing a large number of DNA samples through automation. Thus, since Kubo

teaches each of the limitations of the instant claims, Kubo anticipates the claimed invention.

10. Claims 1, 3, 6-7 are rejected under 35 U.S.C. 102(b) as being anticipated by Ide et al (Biochemistry, Vol 32, pages 8276-8283, 1993).

Ide et al (herein referred to as Ide) teaches a method for detection of aabasic sites in DNA. Ide teaches that the aldehyde group in an aabasic site is first modified by a probe bearing biotin residue, call the Aldehyde Reactive Probe (ARP) and then the tagged biotin is quantified in an ELISA-like assay (abstract). Ide teaches that aabasic sites are common lesions in DNA and are considered to be important intermediates in mutagenesis and carcinogenesis. Ide teaches preparing DNA with aabasic sites by treating calf thymus DNA. Duration of the heat treatment varied to introduce different amounts of aabasic sites into DNA (page 8278, col 2). Ide also teaches sampling DNA from HeLa RC355 cells and subjecting the DNA to the ARP assay. The microtiter plates were incubated with 200 uL of DNA solution in each well. The plates were washed and ARP solution was added and allowed to incubate. Then ABC solution was added followed by enzyme substrate solution, ABTS, and finally subjected to OD measurement at 405 nm (page 8279, col 1).

11. Claims 1-3, 5-7 are rejected under 35 U.S.C. 102(b) as being anticipated by Maulik et al. (Nucleic Acids Research, Vol 27, No. 5, pages 1316-1322, October 1999)

or rejected under 35 U.S.C. 102(e) as being anticipated by Makrigiorgos (US Pat. 6,174,680, January 2001).

Maulik and Makrigiogos appears to be equivalent in numerous respects. However, in the event that applicant's perfect their provisional date, such that the 102(b) would be overcome, the 102(e) rejection would also be of record. In the interest of simplicity, the examiner will refer to Makrigiorgos (US Pat 6,174,680, January 2001).

Makrigiorgos teaches a method of assaying for aabasic sites by using FARP (fluorescent aldehyde reactive probe). Makrigiorgos teaches that for chemiluminescence studies, Reacti-Bind NeutrAvidin coated polystyrene plates are used (col 22, lines 65-67)(limitations of Claim 5). Makrigiorgo teaches FARP-trapping of aldehydes and subsequent DNA biotinylation which includes incubated FARP with DNA, removing the non-covalently bound FARP, and subsequently immobilized the FARP-labeled DNA on microplates and then analyzed in chemiluminescent studies. In section 4, col 23, Makrigiorgos teaches chemiluminescence measurement of FARP-trapped aldehydes in calf thymus or plasmid DNA by immobilizing the dsDNA on microplates and analyzed (col 23, lines 50-65).

12. Claims 1-3, 5-10, 14-15 are rejected under 35 U.S.C. 102(a) as being anticipated by Kow et al (Methods, Vol 22, page 164-169, 2000).

Kow et al (herein referred to as Kow) teaches detection of aabasic sites and oxidative DNA base damage using an ELISA-like assay. The assay uses ARP, a biotinylated aldehyde-specific reagent that has been shown to react specifically with the

aldehyde group present in AP sites resulting in biotin-tagged AP sites. The biotin-tagged AP sites can then be determined calorimetrically with an ELISA-like assay using avidin/biotin-conjugated horseradish peroxidase as the indicator enzyme (abstract). Kow teaches that the sensitivity and simplicity of the ARP assay thus make it a valuable method for investigators who are interested in estimating the level of oxidative DNA damage in cells and tissues derived from patients with various age-related diseases or cancers (abstract). Kow specifically teaches that APR DNA standards are generated. Kow also teaches that ARP-treated DNA can be facilitated to bind to the microtiter plate in the presence of Reacti-Bind DNA coating solution (page 166, col 1)(limitation of Claim 5 and Claim 2). With respect to Claims 8-9 and 14-15, Kow teaches oxidative pyrimidine damage with endonuclease III (page 167, col 1-2). To estimate the level of endonuclease II sensitive sites in DNA samples, DNA is treated with excess endonuclease III and contacted with ARP and analyzed. Table 1 also illustrates additional DNA glycosylases that can be used for the estimation of base damage by coupling with the ARP assay including 8-oxoguanin glycosylase from both human and yeast.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the

invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. Claims 1-3, 6-9, 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kubo et al (Biochemistry, Vol. 31, pages 3703-3708, 1992) in view of Nakamura et al. (Cancer Research, Vol. 58, pages 222-225, January 1998) or Makrigiorgos (US Pat. 6,174,680, January 2001).

Kubo et al (herein referred to as Kubo) teaches a "novel, sensitive, and specific assay for aabasic sites, the most commonly produced DNA lesion". Kubo teaches the use of a biotin-tagged reagent specific for the aldehyde groups, called Aldehyde Reactive Probe (ARP). Kubo teaches that "after modification of the aldehyde group with ARP, the biotin-tagged Ap site can then be easily quantitated by the use of avidin/biotin complex technology in an ELISA-like microtiter plate assay" (page 3703, col 2). Kubo teaches a method of binding to an analysis plate (Immulon I microtiter plates) both sample DNA and control DNA (calf thymus DNA containing AP sites) having known aabasic sites (page 3704, col 2). The plates were then washed and ARP reagent was added. Unbound ARP reagent was removed by washing with PBS-Tween (page 3704, col 2). Finally, ABC was added to each well, washed and horseradish peroxidase substrate was added and the color development was stopped after an appropriate time (limitations of Claim 6). Absorbance at 490 nm was taken and standard curves were determined with either f1 or calf thymus DNA containing known amounts of AP sites (limitations of Claims 1 and 3). Table 1 illustrates the specificity of the ARP reagent and illustrates that the absorbance is greater with the greater number of AP sites. Kubo clearly teaches "microtiter plates coated with calf thymus DNA containing each of these

lesions were incubated with the ARP reagent, and the mount of biotin bound in each of the wells was measured by enzyme assay using the avidin/biotin complex (ABS) conjugated with horseradish peroxidase" (Page 3705, col 1). With respect to Claims 8-9, 14-15, Kubo teaches an additional method for determining the number of intermediary AP sites produced by the action of endonuclease III. The method comprised using 200 ul of calf thymus DNA containing different amount of thymine glycol which was added to each of the wells of a microtiter plate, incubated and washed. Then endonuclease III was added and the plates were incubated. The reaction was terminated and ARP reagent was added and the number of AP sites was determined. The results are illustrated in Figure 4. The ARP assay is rapid, simple, specific and sensitive and offers the possibility of processing a large number of DNA samples through automation.

Kubo does not specifically teaches a method wherein the sample and control DNA are tagged or labeled with ARP reagent prior to being bound to the analysis plate for comparison.

However, Nakamura teaches a method of detecting AP sites using an aldehyde reactive probe-slot-blot assay. The method of Nakamura, to measure AP sites, incubates DNA with ARP prior to any immobilization to a solid support. Nakamura then heat denatures the DNA and immobilizes the single-stranded DNA on a BAS-85 NC membrane.

Additionally, Makrigiorgos teaches a method of using FARP, (fluorescent aldehyde reactive probe). Makrigiorgos teaches FARP-trapping of aldehydes and

subsequent DNA biotinylation which includes incubated FARP with DNA, removing the non-covalently bound FARP, and subsequently immobilizing the FARP-labeled DNA on microplates and then analyzed in chemiluminescent studies. Makrigiorgos teaches chemiluminescence measurement of FARP-trapped aldehydes in calf thymus or plasmid DNA by immobilizing the dsDNA on microplates and analyzed (col 23, lines 50-65).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kubo which teaches immobilizing the DNA to the analysis plate, solid support, prior to contacting the DNA with ARP, with the teachings of Nakamura or Makrigiorgos which incubates the ARP with the DNA prior to the immobilizing step. The ordinary artisan would have recognized that a method which contacted the DNA with the ARP prior to binding to a solid support and a method which contacted the DNA with the ARP following binding to a solid support would have been equivalent methods. Both of the methods provide highly sensitive results to determining the number of AP sites in a sample DNA. Since the art teaches methods which illustrate that DNA and ARP may be contacted both prior and following binding to a solid support, the ordinary artisan would have recognized that it is irrelevant whether the DNA and the ARP are contacted prior or following binding to a solid support.

14. Claims 1, 3-4, 6-9, 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kubo et al (Biochemistry, Vol. 31, pages 3703-3708, 1992) in view of

Nikiforov et al (US Pat. 5,679,524, October 1997) or Wood et al (US Pat. 6,277,570, August 21, 2001).

Kubo et al (herein referred to as Kubo) teaches a "novel, sensitive, and specific assay for aabasic sites, the most commonly produced DNA lesion". Kubo teaches the use of a biotin-tagged reagent specific for the aldehyde groups, called Aldehyde Reactive Probe (ARP). Kubo teaches that "after modification of the aldehyde group with ARP, the biotin-tagged Ap site can then be easily quantitated by the use of avidin/biotin complex technology in an ELISA-like microtiter plate assay" (page 3703, col 2). Kubo teaches a method of binding to an analysis plate (Immulon I microtiter plates) both sample DNA and control DNA (calf thymus DNA containing AP sites) having known aabasic sites (page 3704, col 2). The plates were then washed and ARP reagent was added. Unbound ARP reagent was removed by washing with PBS-Tween (page 3704, col 2). Finally, ABC was added to each well, washed and horseradish peroxidase substrate was added and the color development was stopped after an appropriate time (limitations of Claim 6). Absorbance at 490 nm was taken and standard curves were determined with either f1 or calf thymus DNA containing known amounts of AP sites (limitations of Claims 1 and 3). Table 1 illustrates the specificity of the ARP reagent and illustrates that the absorbance is greater with the greater number of AP sites. Kubo clearly teaches "microtiter plates coated with calf thymus DNA containing each of these lesions were incubated with the ARP reagent, and the mount of biotin bound in each of the wells was measured by enzyme assay using the avidin/biotin complex (ABS) conjugated with horseradish peroxidase" (Page 3705, col 1). With respect to Claims 8-

9, 14-15, Kubo teaches an additional method for determining the number of intermediary AP sites produced by the action of endonuclease III. The method comprised using 200 ul of calf thymus DNA containing different amount of thymine glycol which was added to each of the wells of a microtiter plate, incubated and washed. Then endonuclease III was added and the plates were incubated. The reaction was terminated and ARP reagent was added and the number of AP sites was determined. The results are illustrated in Figure 4. The ARP assay is rapid, simple, specific and sensitive and offers the possibility of processing a large number of DNA samples through automation.

Kubo does not specifically teach performing simultaneous assays for the sample DNA and the control DNA so as to remove environmental or process variables at the comparing step.

However, Nikiforov et al (Nikiforov) teaches that 96-well microtiter dishes used in diagnostic laboratories allow the simultaneous determination of a large number of samples and controls.

Similarly, Wood et al (herein referred to as Wood) teaches the 96 well microtiter plate format allows for simultaneous processing of multiple patient samples. Wood teaches twelve wells are occupied by controls, and samples may be found in the other wells.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified method of Kubo to use several wells for the control DNA and several wells for the sample DNA as taught by Nikiforov

or Wood. The ordinary artisan would have clearly recognized the importance of controls and would have recognized that subjecting the controls to the same conditions as the test samples would have the expected benefit of controlling for variables which may have changed between runs, for example temperature, pressure or other additional changes. Further, running controls in the same microtiter plate simultaneously has been used in the art routinely. Nikiforov, in 1994, recognized the importance and benefits of running samples and controls simultaneously. In addition to removing environmental or process variables, which is the concept behind controls, running controls simultaneous with the tests samples provides the expected benefit of reducing time, and materials needed for the assay. The ordinary artisan would have been motivated to have consolidated the experiments taught by Kubo into a single well assay for the benefit of controlling for variations in conditions and processes in addition to the benefit of saving time and reagents.

15. Claims 1-3, 5-10, 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kubo et al (Biochemistry, Vol. 31, pages 3703-3708, 1992) in view of Makrigiorgos (US Pat. 6,174,680, January 2001).

Kubo et al (herein referred to as Kubo) teaches a "novel, sensitive, and specific assay for abasic sites, the most commonly produced DNA lesion". Kubo teaches the use of a biotin-tagged reagent specific for the aldehyde groups, called Aldehyde Reactive Probe (ARP). Kubo teaches that "after modification of the aldehyde group with ARP, the biotin-tagged Ap site can then be easily quantitated by the use of avidin/biotin

complex technology in an ELISA-like microtiter plate assay" (page 3703, col 2). Kubo teaches a method of binding to an analysis plate (Immulon I microtiter plates) both sample DNA and control DNA (calf thymus DNA containing AP sites) having known abasic sites (page 3704, col 2). The plates were then washed and ARP reagent was added. Unbound ARP reagent was removed by washing with PBS-Tween (page 3704, col 2). Finally, ABC was added to each well, washed and horseradish peroxidase substrate was added and the color development was stopped after an appropriate time (limitations of Claim 6). Absorbance at 490 nm was taken and standard curves were determined with either f1 or calf thymus DNA containing known amounts of AP sites (limitations of Claims 1 and 3). Table 1 illustrates the specificity of the ARP reagent and illustrates that the absorbance is greater with the greater number of AP sites. Kubo clearly teaches "microtiter plates coated with calf thymus DNA containing each of these lesions were incubated with the ARP reagent, and the mount of biotin bound in each of the wells was measured by enzyme assay using the avidin/biotin complex (ABS) conjugated with horseradish peroxidase" (Page 3705, col 1). With respect to Claims 8-9, 14-15, Kubo teaches an additional method for determining the number of intermediary AP sites produced by the action of endonuclease III. The method comprised using 200 ul of calf thymus DNA containing different amount of thymine glycol which was added to each of the wells of a microtiter plate, incubated and washed. Then endonuclease III was added and the plates were incubated. The reaction was terminated and ARP reagent was added and the number of AP sites was determined. The results are illustrated in Figure 4. The ARP assay is rapid, simple, specific and

sensitive and offers the possibility of processing a large number of DNA samples through automation.

Kubo does not specifically teach using Reacti-bind during the binding step.

However, Makrigiorgos teaches a method of detecting abasic sites in DNA using Reacti-Bind NeutrAvidin coated polystyrene plates (col 22, lines 65-67). Makrigiorgos teaches that the Reacti-Bind NeutrAvidin plates are used in chemiluminescence studies.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the microtiter plates of Kubo with alternative microtiter plates which were coated with Reacti-Bind NeutrAvidin. The ordinary artisan would have been motivated to have used the microtiter plates taught by Markigiorgos because Markigiorgos taught that the Reacti-Bind coated plates were useful for chemiluminescence studies.

Conclusion

16. No claims allowable over the art.

17. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Friday 7:00 a.m. to 4:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

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February 13, 2002 JG

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